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Effects of chronic treatment with a novel angiotensin converting enzyme inhibitor, CS622, and a vasodilator, hydralazine, on atrial natriuretic factor (ANF) in spontaneously hypertensive rats (SHR)

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SUMMARY: We studied the effects of chronic treatment with a novel angiotensin converting enzyme inhibitor, α-[(2S,6R)-6-[(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino-5-oxo-2-(2-thienyl)perhydro-1,4-thiazepin-4-yl]acetic acid·HCl (CS622), and a vasodilator, hydralazine, on plasma atrial natriuretic factor (ANF) levels and kidney ANF receptors in spontaneously hypertensive rats (SHR). Plasma ANF level was decreased and cardiac hypertrophy reduced in CS622 treated SHR, but not in hydralazine treated SHR, although blood pressure was lowered similarly in both SHR groups. The binding capacity of kidney ANF receptors increased and the affinity decreased in CS622 treated SHR compared to untreated SHR. These results suggest that decrease of plasma ANF results from decreased cardiac load but not from lowered blood pressure, and that changes in ANF receptors result from increased plasma ANF.

Mammalian cardiac atria synthesize and store vasoactive, diuretic and natriuretic peptides, which are known as atrial natriuretic factors (ANFs) (1,2). ANF is released into the circulation in response to atrial stretching (3). It also inhibits aldosterone secretion and decreases renin secretion (4-7). It has been suggested that ANF is involved in the regulation of extracellular fluid volume, electolyte balance and blood pressure (8). Many investigators have measured concentration of plasma ANF in experimental hypertensive rats (9-16). Plasma levels of ANF in spontaneously hypertensive rats (SHR) have been shown to increase with the development of hypertension in comparison to age-matched Wistar Kyoto rats

ABBREVIATIONS: ANF, atrial natriuretic factor; PMSF, phenylmethyl sulphonyl fluoride; ACE, angiotensin converting enzyme; SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats

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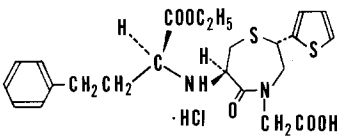

Figure 1. Chemical structure of α-[(2S,6R)-6-[(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino-5-oxo-2-(2-thienyl)perhydro-1,4-thiazepin-4-yl]acetic acid·HCl (CS622)

(WKY) (10). The increase of plasma ANF levels in SHR is probably due to an increase of ANF release caused by cardiac load, elevation of blood pressure or volume expansion (3).

Binding sites for ANF have been identified in many tissues (17-19). It has been reported that the binding capacity of ANF receptors in SHR kidney is lower than that in WKY (20-22). However, mechanisms underlying these changes, and the relationship between changes in ANF levels and ANF receptor, are not clear at present. To delineate this point, we studied changes in plasma ANF and ANF receptor characteristics in SHR chronically treated with two different antihypertensive agents: an ACE inhibitor, CS622 (Fig.1)(23), and a vasodilator, hydralazine.

MATERIALS AND METHODS

Male SHR and WKY, age 20 week-old, were purchased from HOSHINO Laboratory Animals (Japan). Food and water were administered ad libitum. The animals were treated with drugs for 21 weeks, and blood pressure was measured every 3 weeks by the tail-cuff method. SHR were divided into 3 groups: the CS622 group which was treated with CS622 (10 mg/kg/day, p.o. dissolved in 0.3% CMC), the hydralazine group which was treated with hydralazine (5 mg/kg/day, p.o. in drinking water), and the control group which was given 0.3% CMC. At the end of dosing, blood was taken from the abdominal aorta under light anesthesia with ether, and collected into tubes which contained protease inhibitors and cooled in crushed ice. The protease inhibitors included EDTA (1 mg/ml), aprotinin (500 KIU/ml), PMSF (10μM), pepstatin (5μM) and soybean trypsin inhibitor (50 BAEE units/ml) (10). The plasma was separated by centrifugation at 4°C and stored at -100°C. Extraction of plasma ANF using Sep-Pak C_{18} (Waters) was performed according to the methods described by Lang et al.(3). Atrial homogenate was prepared as follows (24). The atria were removed immediately after blood sampling and stored at -100°C until extraction. The atria were boiled for 5 minutes in 10 volumes of 1 M acetic acid containing 20 mM HCl to abolish intrinsic proteolytic activity and homogenized with a Polytron homogenizer. The homogenate was centrifuged at 15,000 x g for 30 minutes at 4°C, and the supernatant was stored at -100°C until radioimmunoassay (RIA). ANF was measured by RIA using a commercial kit (Amersham).

Kidney cortex membrane fraction was prepared according to Napier et al.(17). Kidneys were rapidly removed and placed in cold physiological saline buffer. The cortex was dissected from the kidney and homogenized

with a Polytron in cold buffer containing 1 mM $NaHCO_3$ and 0.1% bacitracin (pH 7.5). The homogenate was centrifuged at 1,000 x g for 30 seconds at 4°C, and the supernatant was centrifuged at 100,000 x g for 60 minutes at 4°C. The pellets were resuspended and washed by centrifugation. Final pellets were resuspended in the buffer. Protein concentration was determined by the Coomassie brilliant blue G 250 dye-binding method with bovine serum albumin as standard. Aliquots (0.1 mg protein/tube) of the membrane fraction were incubated in duplicate for 60 minutes at 4°C as described by Napier et al. (17) in the presence of increasing concentrations of unlabelled ANF (10^{-11} -10^{-6}M; Peptide Institute Inc.) and 50 pM [^{125}I]ANF (Amersham) in a final volume of 0.1 ml. Membrane bound [^{125}I]ANF was separated by rapid filtration on a polyethyleneimine-treated Whatman GF/C followed by three washings with 0.5 ml of cold buffer. [^{125}I] ANF retained on filters were then counted in a gamma counter. Data were expressed as means ± SEM. Statistical difference was calculated by Student's t test for single comparison or one-way analysis of variance combined with Scheffe's analysis for multiple comparison. Scatchard plots for binding data were determined by linear regression analysis.

RESULTS

Table 1 shows body weight, systolic blood pressure and relative heart weight in all groups studied. Systolic blood pressure of the CS622 group and the hydralazine group were significantly lower than that of the control group. Relative heart weight was reduced in the CS622 group, but not in the hydralazine group. As shown in Table 2, CS622 decreased plasma ANF concentration, whereas hydralazine had no significant effect. Taking all the groups together, a good correlation was found between plasma ANF and relative heart weight ($R=0.62$; $p<0.001$), whereas no significant correlation was found between systolic blood pressure and plasma ANF. No differences were observed in atrial ANF content in any of the groups. Table 3 shows the results of Scatchard analysis of the [^{125}I]ANF binding to the rat kidney

Table 1. EFFECTS OF CHRONIC TREATMENT WITH CS622 AND HYDRALAZINE ON BLOOD PRESSURE, BODY WEIGHT AND HEART WEIGHT IN SHR

Group	Blood Pressure (mmHg)	Body Weight (g)	Heart Weight (mg/100g body weight)
Control (n=10)	209 ± 4.6	416 ± 7.8	438 ± 12
CS622 (n=10)	173 ± 3.4**	439 ± 7.0	369 ± 9**
Hydralazine (n=9)	128 ± 3.4**	430 ± 9.2	388 ± 14
WKY (n=9)	138 ± 3.4**	407 ± 11.3	324 ± 17**

Values are means ± SEM.
**$p<0.01$, significantly different from control SHR.

Table 2. EFFECTS OF CHRONIC TREATMENT WITH CS622 AND HYDRALAZINE ON PLASMA ANF AND ATRIAL ANF CONTENT IN SHR

Group	Plasma ANF (pg/ml)	Atrial ANF (μg/g)
Control (n=10)	996.7 ± 102.7	58.4 ± 4.6
CS622 (n=10)	482.1 ± 71.6**	59.3 ± 5.0
Hydralazine (n=9)	921.4 ± 86.0	59.8 ± 4.7
WKY (n=9)	596.1 ± 54.8**	60.0 ± 4.0

Values are means ± SEM.
**p<0.01, significantly different from control SHR.

cortex membrane in the CS622 group. The binding capacity (Bmax) for ANF was higher in the CS622 group than in the control group and the receptor affinity (Kd) was significantly lower in the CS622 group.

DISCUSSION

In the present study, we have shown that chronic treatment with a novel ACE inhibitor, CS622, reversed cardiac hypertrophy and decreased plasma ANF concentration, whereas treatment with hydralazine had no significant difference in plasma ANF and relative heart weight despite the lowered systolic blood pressure.

There are several reports on the correlation between blood pressure and plasma ANF concentrations in experimental hypertensive rats. Gutkowska et al. (10) found that plasma ANF levels in SHR increase with the development of hypertension in comparison to age-matched WKY. Other investigators, using SHR (9,11), DOCA-NaCl rats (12,13) and 1K-1C rats

Table 3. ANF RECEPTORS IN CS622-TREATED AND UNTREATED SHR

Group	Kd (pM)	Bmax (fmol/mg protein)
Control (n=10)	519.1 ± 66.8	29.27 ± 4.6
CS622 (n=10)	831.2 ± 59.0**	42.98 ± 4.2*

Kd = dissociation constant. Bmax = maximum binding capacity.
Values are means ± SEM.
**p<0.01, *p<0.05, significantly different from control SHR.

(14), showed that plasma ANF levels in experimental hypertensive rats are higher than that in normotensive rats. These increases in plasma ANF may result from stimulation of ANF release from the atria. The increased ANF release was apparently related to increased arterial blood pressure, fluid volume and atrial filling pressure (3). R. Garcia et al. (13) demonstrated that the release of ANF in experimental hypertension depends on the pathogenesis, and could be related either to blood pressure or blood volume expansion.

In the present study, we found that treatment with hydralazine had no significant effect on plasma ANF level although the agent lowered systolic blood pressure. Moreover, there was no significant correlation between systolic blood pressure and plasma ANF. These results suggest that elevated blood pressure per se was not the major factor in ANF release in SHR.

Stasch et al.(25) investigated the effect of chronic treatment with a calcium antagonist, nitrendipine, in SHR, and found a correlation between plasma ANF and relative heart weight. They noted that the changes in plasma ANF levels were secondary to the hypertensive disease and the associated increase in cardiac load. Garcia et al.(13) also found a similar correlation and suggested that the ventricles might participate in ANF release. In the present study, a significant correlation was found between relative heart weight and plasma ANF. Hence, the decrease of plasma ANF level in CS622 treated SHR is possibly due to cardiac load reduction.

We have to consider other factors such as angiotensin II level, sympathetic nerve activity and blood volume. Angiotensin II is known to stimulate the ANF release in rats (26). CS622 decreases plasma angiotensin II concentration by ACE inhibition whereas hydralazine increases it (23,27). Hydralazine increases blood volume as a result of sodium and water retention and increases sympathetic nerve activity (27). On the other hand, an ACE inhibitor, captopril, has been reported to suppress sympathetic nerve activity and not to increase blood volume on chronic dosing (28,29).

Therefore, these factors could also be involved in the differential regulation of ANF release produced by CS622 and hydralazine.

Binding sites for ANF have been identified in several tissues using membrane binding and autoradiographic technique (17-22). Some investigators have found that glomerular Bmax and Kd are decreased in experimental hypertensive rats compared to the normal rats (20,21). They have related the down-regulation of renal glomerular ANF receptors to the increase of plasma ANF level, and stated that the increased affinity for ANF binding may represent a compensatory response to the lowered Bmax (21,22). The exact opposite was found in the present study when blood pressure of SHR was kept lowered by long-term treatment with CS622 : decreased plasma ANF concentration, increased Bmax and Kd, which mean increased binding capacity and decreased affinity to the receptors.

In conclusion, long-term treatment with CS622 but not with hydralazine decreased plasma ANF levels, and increased Bmax and Kd of renal ANF receptors. The decrease of plasma ANF may be attributed either to a decrease in cardiac load or decreased angiotensin II levels, but not to lowered blood pressure. Changes in ANF receptor characteristics are secondary to the decrease of plasma ANF.

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